Impact of Caramelization on the Glass Transition Temperature of Several Caramelized Sugars. Part II: Mathematical Modeling

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Further to part I of this study, this paper discusses mathematical modeling of the relationship between caramelization of several sugars including fructose, glucose, and sucrose and their glass transition temperatures (T_g). Differential scanning calorimetry (DSC) was used for creating caramelized sugar samples and determining their glass transition temperatures (T_g). UV−vis absorbance measurement and high-performance liquid chromatography (HPLC) analysis were used for quantifying the extent of caramelization. Specifically, absorbances at 284 and 420 nm were obtained from UV−vis measurement, and the contents of sucrose, glucose, fructose, and 5-hydroxymethyl-furfural (HMF) in the caramelized sugars were obtained from HPLC measurements. Results from the UV and HPLC measurements were correlated with the T_g values measured by DSC. By using both linear and nonlinear regressions, two sets of mathematical models were developed for the prediction of T_g values of sugar caramels. The first set utilized information obtained from both UV−vis measurement and HPLC analysis, while the second set utilized only information from the UV−vis measurement, which is much easier to perform in practice. As a caramelization process is typically characterized by two stages, separate models were developed for each of the stages within a set. Furthermore, a third set of nonlinear equations were developed, serving as criteria to decide at which stage a caramelized sample is. The models were evaluated through a validation process.

KEYWORDS: Glass transition; modeling; caramelization; sugar; differential scanning calorimetry; UV−visible spectrometry; high-performance liquid chromatography

INTRODUCTION

Many processed solid food materials exist in an amorphous (i.e., glass) or partially amorphous state where only short-range molecular arrangement exists with the absence of long-range molecular packing (*1*). The glass transition temperature (T_g) can be regarded as a point or a temperature range around the point, where some of the physical and chemical properties of the material undergo changes. It has been well-accepted that T_g is an important parameter for predicting and even controlling food stability during processing and storage (*2*). Among several instrumental methods, differential scanning calorimetry (DSC) has been the most commonly used one for determining T_g values.

As demonstrated in part I of this study (*3*), caramelization of a sugar changes the chemical composition of the sugar, which can result in a considerable change in its T_g value. Thus, for a food product with high sugar content, caramelization may significantly affect the T_g value of the product and, therefore, its stability during processing and storage. There is a need to be able to effectively determine or predict the T_g value brought by caramelization.

Meanwhile, although the T_g values of various types of amorphous sugar have been reported in the literature (*4–6*), there are considerable differences among the values by different authors for the same type of sugar, for example, sucrose. Sugar caramelization during the course of measuring T_g by DSC could be one of the major reasons for those differences. It was demonstrated in ref *7* that the T_g value depended strongly on the condition of melting sucrose crystals—an essential step in creating amorphous sucrose in DSC. However, until now, only the impact of caramelization on T_g of sucrose was assessed qualitatively. Besides our study as described in part I (*3*), there has been no report on other commonly used sugars such as glucose and fructose. Furthermore, no valid method has been developed to quantitatively assess the impact.

Mathematical modeling is a powerful tool for establishing quantitative relationships among various physical and chemical parameters. Models can be developed by a variety of approaches including linear and nonlinear regressions, fuzzy logic, artificial

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Table 1. List of Independent Variables x_1 to x_6

variables	values
x_1	A_{284}/sample weight
x_2	A_{420}/sample weight
x_3	μg fructose/sample weight
x_4	μg glucose/sample weight
x_5	μg sucrose/sample weight
x_6	μg HMF/sample weight

Table 2. Equations for the Derived Variables x_7 to x_{10}

sugar	variable	equation
fructose	x_7	$-6.30 \ \text{Ln} \ x_1 - 5.69$
	x_8	$32.49 \ e^{-0.55x_1} - 21$
	x_9	x_2/x_1
	x_{10}	$55.00 - 65.17 \ e^{50.64(-x_2/x_1)}$
glucose	x_7	$-3.76 \ \text{Ln} \ x_1 + 15.01$
	x_8	$29.14 \ e^{-1.19x_1}$
	x_9	x_2/x_1
	x_{10}	$150.00 - 132.84 \ e^{41.23(-x_2/x_1)}$
sucrose	x_7	$-12.14 \ \text{Ln} \ x_1 + 38.39$
	x_8	$59.72 \ e^{-0.27x_1}$
	x_9	x_2/x_1
	x_{10}	$95.00 - 73.23 \ e^{44.36(-x_2/x_1)}$

Table 3. Variables Used in the Final Models

model set	stage of caramelization	variables selected by stepwise regression
set 1	stage 1	$x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8$
	stage 2	$x_1, x_2, x_3, x_4, x_5, x_6, x_9, x_{10}$
set 2	stage 1	x_7, x_8
	stage 2	x_{10}

neural networks, etc. Not only the models can provide much insight to the underlying relationship among various parameters, but also certain parameters that cannot be measured easily (such as T_g) could be calculated from the models utilizing other parameters that are relatively easier to obtain (such as UV−vis absorbance).

The objective of this research was to study the changes in T_g of three types of amorphous sugars due to caramelization and to provide a quick and reliable method for predicting the T_g value of a caramelized sugar sample. A previous paper on part I of this study (*3*) described how the glass transition temperature changed with heating temperature and holding time, impacted by the formation of small molecules during initial sugar degradation and large molecules in later stage of caramelization. This paper presents mathematical models that were developed

based on the results and analysis in part I and demonstrates that the models enabled us to predict the T_g of a caramelized sugar using parameters that can be measured easily and accurately.

MATERIALS AND METHODS

Materials. Anhydrous and high-performance liquid chromatography (HPLC) grade sugars including glucose (Aldrich, Steinheim, Germany), fructose (Sigma, St. Louis, MO), and sucrose (AnalaR, Poole, England) were purchased. HPLC grade 5-(hydroxymethyl)furfural (HMF) of 99% purity was purchased from Aldrich (Steinheim, Germany).

Experimental Methods. Details of the experimental methods for creating caramelized sugars, measuring T_g values, and quantifying the extent of caramelization can be found in part I of this study (*3*). Briefly, sugar samples were first vaccuum-dried, and then, they were caramelized under various heating and holding conditions in a DSC (Mettler Toledo, DSC 821^e, Schwerzenbach, Switzerland), and the corresponding T_g values of the caramelized samples were measured by the same DSC. After the DSC treatment and measurement, the samples were dissolved in deionized water and the absorbance at 284 and 420 nm were measured by an UV−vis spectrometer (Shimadzu UV-1700, Kyoto, Japan). Sucrose, glucose, fructose, and HMF of the samples were quantified using a HPLC system (Shimadzu, LC-10Avp) with refractive index and photodiode array detectors.

Mathematical Modeling. Experiments for fructose with a final heating temperature of 160 °C at various holding times were carried out in triplicate, while all other experiments were in duplicate. As shown in part I of this study (*3*), even using a DSC under the same heating profile, there were considerable variations and inconsistencies in the caramelized samples created, due to the highly complicated nature of caramelization reactions. In other words, samples repeated under the same heating condition did not necessarily yield similar caramelization. Therefore, during the modeling process, all experimental results in replicates were taken into account individually rather than an average of the replicates being taken.

A total of 10 variables were used for modeling T_g, including six independent variables and four derived variables. As listed in **Table 1**, the six independent variables x_1−x_6 are the normalized UV−vis absorbance A_{284} (x_1) and A_{420} (x_2), and the concentrations of fructose (x_3), glucose (x_4), sucrose (x_5), and HMF (x_6) measured by HPLC. Normalized values were obtained by dividing a measured value by the respective sample weight in milligrams. From now on, A_{284} and A_{420} refer to normalized values. Sugar caramelization can be roughly divided into two stages, namely, decomposition and polymerization (*3, 8, 10*). The first stage is dominated by the break down of sugar to small molecules by dehydration, bond cleavage, retro-aldolization, etc. In the second stage, more brown-colored polymeric substances are formed via radical polymerization, which is the predominating reaction in this stage (*10*). For modeling the first stage of caramelization, the independent variable A_{284} (i.e., x_1) was correlated with T_g by nonlinear regression. The correlation was carried out by finding two best-fitted functions between T_g and A_{284}, $y_1 = f_1(x_1)$ and $y_2 = f_2(x_1)$; the values of

Table 4. Developed Models for T_g Prediction

sugar	stage	model no.	models	R^2	RMSE
fructose	1	F1.a	$T_g = 28.72 \ e^{-0.55x_1} - 19.38$	0.9718	1.56
		F1.b	$T_g = 0.88x_8 - 0.82 = 28.72 \ e^{-0.55x_1} - 19.38$	0.9718	1.56
	2	F2.a	$T_g = -1.65x_6 + 0.54x_4 - 0.25x_3 + 0.08x_5 + 55.88$	0.9033	6.04
		F2.b^a	$T_g = 1.13x_{10} - 8.84 = 53.29 - 73.63 \ e^{-50.64(x_2/x_1)}$	0.3402	16.82
glucose	1	G1.a	$T_g = -3.18 \ \text{Ln} \ x_1 - 0.08x_3 + 14.39(x_2/x_1) + 17.10$	0.9942	0.42
		G1.b	$T_g = x_7 = -3.76 \ \text{Ln} \ x_1 + 15.01$	0.9893	0.56
	2	G2.a	$T_g = -105.01 \ e^{-41.23(x_2/x_1)} - 0.21x_4 + 147.50$	0.9697	5.95
		G2.b	$T_g = 1.09x_{10} - 10.66 = 152.84 - 144.80 \ e^{-41.23(x_2/x_1)}$	0.9500	8.71
sucrose	1	S1.a	$T_g = -0.21x_6 - 10.53 \ \text{Ln} \ x_1 + 0.03x_4 + 32.70$	0.9983	0.75
		S1.b	$T_g = 0.57x_7 + 0.51x_8 - 2.78 = -7.17 \ \text{Ln} \ x_1 + 30.40 \ e^{-0.27x_1} + 19.12$	0.9971	0.99
	2	S2.a	$T_g = -4.05x_6 + 50.49 \ e^{-44.36(x_2/x_1)} + 0.24x_1 - 224.80(x_2/x_1) + 64.85$	0.9932	1.35
		S2.b	$T_g = 1.24x_{10} - 19.25 = 98.40 - 90.70 \ e^{-44.36(x_2/x_1)}$	0.8765	5.71

^{a} This particular model (F2.b) is of very poor quality because of its extremely low R^2 value (0.3402), indicating that there was no significant correlation among the variables.

Table 5. Criterion Equations for Deciding the Stage of Caramelization

sugar	stage	criterion equation
fructose	1	$Y = -585.25x_2 + 0.37$
	2	$Y = 19.73 \ln x_2 + 58.80$
glucose	1	$Y = <23.4.96 \ln x_2 - 7.10$
	2	$Y = 34.43 \ln x_2 + 211.61$
sucrose	1	$Y = <23.14.57 \ln x_2 - 32.55$
	2	$Y = 22.29 \ln x_2 + 108.10$

y_1 and y_2 were calculated and labeled as x_7 and x_8. For modeling the second stage of caramelization, a new variable was derived using A_{420}/A_{284} and was labeled as x_9. T_g was correlated with x_9, again by nonlinear regression, and a best-fitted function $y_3 = f_3(x_9)$ was obtained. y_3 thus obtained was labeled as x_{10}. $x_7 - x_{10}$ were the four derived variables developed for modeling, and their details are listed in **Table 2**.

Variables $x_1 - x_6$ were combined with $x_7 - x_{10}$ for developing mathematical models to predict the T_g values of caramelized sugars. Two sets of models were developed for T_g prediction; one set utilized the results of both UV and HPLC measurements (set 1), and the other one utilized only the results from UV measurement (set 2).

The models were developed using multiple linear regressions (MLR). The appropriate variables to be used in the final models were selected by stepwise regression, with both forward selection and backward elimination to sequentially introduce the variables into the models one at a time. **Table 3** lists the finally selected variables in the developed models.

To be able to apply a correct model to a sample, it has to be decided at what stage of caramelization the sample is. For this purpose, T_g was correlated with x_2 (i.e., A_{420}) by nonlinear regression for each stage of caramelization. The correlation equations were used as the criteria for deciding the stage of caramelization in which a caramelized sugar was.

To validate the models, the T_g values of all of the samples were calculated using the models and compared to the measured T_g. Furthermore, additional samples under new caramelization conditions were also created for validation. The accuracy of the prediction was evaluated by the coefficient of determination (R^2) as well as the root-mean-square error (RMSE) between the predicted T_g values and the measured T_g values.

RESULTS AND DISCUSSION

Model Development. Mathematical models were developed for a better understanding of the effects of caramelization on the glass transition temperature of sugars, as well as for providing an accurate prediction of the glass transition temperature of caramelized sugars.

Because of the significantly different nature of the predominant reactions between the first and the second stages of caramelization, the change of T_g was also divided into two stages, that is, decomposition and polymerization. In the first stage, the T_g generally deceased with time, and in the second stage, the T_g generally increased with time, and models were developed separately for each stage. Two sets of models were developed for T_g prediction, with one set utilized the results from both UV and HPLC measurements (set a), and the other one used only the results from UV measurement (set b).
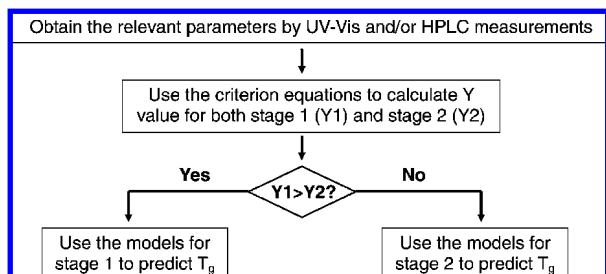
Figure 1. Decision tree for T_g prediction.
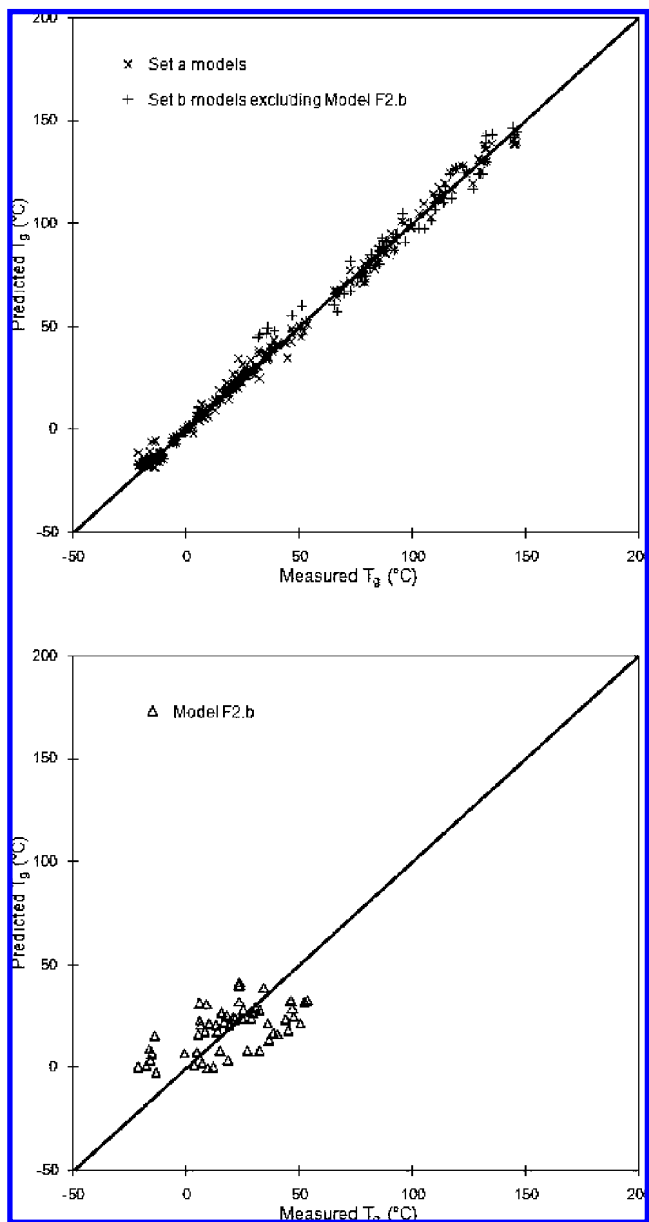
Figure 2. Predicted T_g vs measured T_g using the criteria for choosing the stage of caramelization and the corresponding model. Top, all models excluding model F2.b; bottom, model F2.b.

To correlate T_g with selected variables, MLR was carried out and the optimum subsets of variables to be used in the models were determined by stepwise regression. The resulted nonlinear models describing the influence of caramelization on T_g involving both UV and HPLC measurements are shown in **Table 4** (models with affix "a").

It was found during the model development that variables x_7 and x_8 had good correlation with T_g in the first stage of caramelization, and variables x_9 and x_{10} had good correlation with T_g in the second stage of caramelization (data not shown). A new set of models for the T_g prediction were therefore developed by MLR using only x_7, x_8, x_9, and x_{10} for the two stages (models with affix "b" in **Table 4**).

Criteria for Deciding the Stage of Caramelization. A separate set of equations were developed as the criteria for deciding the stage of caramelization and thus the corresponding model to be used for T_g prediction. **Table 5** lists the equations developed from correlating T_g to x_2 in the two stages of caramelization. It was found that for a sugar, at any stage, the

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Table 6. Model Validation Using New Samples

sample	measured T_g (°C)	set a		set b	
		predicted T_g (°C)	difference (°C)	predicted T_g (°C)	difference (°C)
fructose 1	12.01	8.48	3.53	11.44	0.57
fructose 2	46.52	49.86	−3.34	29.72[a]	16.80[a]
glucose 1	39.28	37.50	1.78	40.02	−0.74
glucose 2	66.43	60.83	5.60	69.15	−2.72
sucrose 1	70.02	71.05	−1.03	71.38	−1.36
sucrose 2	74.59	71.53	3.06	83.70	9.11

[a] Data not valid due to the invalid model.

equation of that stage always gave the larger value among the two. The procedure of using the criteria and models to predict T_g values is illustrated in a decision tree as shown in **Figure 1**.

Comparison of the Two Sets of Models. The usefulness of the two sets of models was compared by their power of prediction and accuracy based on their R^2 and RMSE values (**Table 4**). Large R^2 values and small RMSE values are desired for a high-quality model. Except for model F2.b, all of the models in **Table 4** were of relatively good quality, indicated by large R^2 values and small to reasonable RMSE values.

Comparison of the set a models and set b models showed that R^2 values of the set a models were always higher than those of the set b models. In the first stage of caramelization, the differences in R^2 values between the two sets of models were marginal, while in the second stage of caramelization, the differences became bigger. While model G2.b and model S2.b were still acceptable, model F2.b showed a R^2 value of 0.3402, which means that the correlation between T_g and x_{10} was poor and the UV/vis measurement alone could not characterize the change of T_g of the caramelized fructose.

Although the set a models are more accurate as indicated by their larger R^2 and smaller RMSE values, it is recommended that the set b models can be used for T_g prediction in the first stage of caramelization because of its simplicity and reasonable accuracy. Because the set b models for glucose and sucrose in the second stage of caramelization (i.e., model G2.b and model S2.b) are, although still acceptable, significantly less accurate than those of the set a models, their use is only recommended when HPLC is not available.

Model Validation. The predicted T_g values were plotted against the measured T_g to verify the performance of the models, shown in **Figure 2**. It can be seen that the T_g values predicted using the set a models were very close to the measured T_g values. The T_g values predicted using the set b models except model F2.b also showed satisfactory performance.

To further validate the two sets of models, two samples of each type of sugar were caramelized under new conditions that were different from those used for modeling. UV−vis and HPLC measurements were carried out as described earlier. Their T_g values were measured using DSC and also predicted according to the criterion equations and models developed. The predicted T_g values were compared with the measured T_g values to evaluate the accuracy of the models, shown in **Table 6**.

It can be seen that the prediction using the set a models involving both UV/vis and HPLC measurements was reasonably accurate, with the largest difference being 5.60 °C (**Table 6**). The set b models, again except for model F2.b, also predicted T_g values reasonably accurately, with the second sucrose sample producing the largest difference of 9.11 °C. Considering that the variation in most T_g measurements could be as large as 12 °C due to different DSC operating conditions (*11*), the accuracy of the model predictions is quite acceptable. Considering the

model accuracy as well as the corresponding experimental effort and time to be involved, except for model F2.b, both sets of models can be used for T_g prediction, depending on the availability of HPLC measurements.

In conclusion, to predict the glass transition temperature of caramelized sugar using simple measurements, the relationship between the properties of caramelized sugar and their corresponding glass transition temperature was studied, and two sets of mathematical models were developed for T_g prediction. The set a models involve both UV/vis and HPLC measurements, and the set b models involve only UV/vis measurement. Within each set of the models, two models were developed for each type of sugar, corresponding to the first and second stages of caramelization, respectively. A separate set of equations were also developed as the criteria for deciding the stage of caramelization, so that correct models can be selected for T_g prediction.

Although the set a models are slightly more accurate than the set b models, except for model F2.b, the accuracy of both sets of models is acceptable, proven by their high R^2 values and small to reasonable RMES values. It is therefore recommended that the set b models (except for model F2.b) be used instead of the set a models for the prediction of T_g, especially in the first stage of caramelization, due to their simplicity.

The mathematical models developed can be used for predicting T_g of a caramelized sugar and, subsequently, could contribute to the design of food products containing high sugar content and their processing and storage conditions. It should be noted that caramelization of different types of sugar impacts the T_g value differently. Thus, all models developed in this study are only valid for their specified type of sugar. On the other hand, for the same type of sugar, the condition of caramelization, such as the final heating temperature and reaction time, will not affect the validity of the models.

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